Amendments to the Specification:

Replace the current sequence listing with the sequence listing submitted herewith.

Amend the paragraph beginning at page 24, line 5, as follows.

By a "DAF-3 polypeptide" is meant a polypeptide that complements (as defined above) a *C. elegans daf-3* mutation and/or that possesses at least 60% amino acid sequence identity to SEQ ID NO: 35, at least 38% amino acid sequence identity to SEQ ID NO: 36, at least 47% amino acid sequence identity to SEQ ID NO: 85, or a combination thereof. Preferably, a DAF-3 polypeptide includes a proline or a glycine at amino acid positions corresponding to *C. elegans daf-3* amino acids at positions 200 (proline) and/or 620 (glycine) in Fig. 12A, respectively, or a combination thereof. For example, the polypeptide may include a proline in the motif GRKGFPHV (~~SEQ ID NO:200~~ SEQ ID NO:322) or a glycine in the motif RXXIXXG (where X is any amino acid) (~~SEQ ID NO:201~~ SEQ ID NO:323).

Amend the paragraph beginning at page 35, line 9, as follows.

Fig. 2A shows the predicted *C. elegans* DAF-2 amino acid sequence (SEQ ID NO:12). The predicted cysteine-rich region (amino acids 207-372) and tyrosine kinase domain (amino acids 1124-1398) are boxed. The signal peptide (amino acids 1-20), proteolysis site (amino acids 806-809), transmembrane domain (amino acids 1062-1085), and PTB binding motif in the juxtamembrane region (NPEY, amino acids 1103-1106)

are underlined. Three DAF-2 tyrosine residues, Y1293, Y1296 and Y1297, in the region corresponding to the insulin receptor kinase Y1158 to Y1163 activation loop are likely to be autophosphorylated, based on the predicted similarity between the DAF-2 and insulin receptor phosphorylation targets (Fig. 2B). Another likely target for DAF-2 autophosphorylation is the Y1106 NPEY motif located in the region corresponding to the insulin receptor juxtamembrane region NPEY motif (at Y972), that has been shown to mediate IRS-1 binding via its PTB domain to the insulin receptor (White and Kahn, *J. Biol. Chem.* 269: 1-4, 1994). While DAF-2 bears one YXXM motif implicated in coupling to PI 3-kinase, mammalian IRS-1 and *Drosophila* insulin receptor (Fernandez et al., EMBO J. 14: 3373-3384, 1995) bear multiple YXXM motifs. Although no p85-like adaptor subunit has yet been detected in the *C. elegans* database, the AGE-1 homology to mammalian p110 suggests the existence of a homologous or analogous adaptor (Morris et al., *Nature* 382: 536-539, 1996). In the DAF-2 C-terminal domain, two other tyrosine residues may be autophosphorylated and bound to particular SH2-containing proteins: Y1678 binding to a PLC-g or SHP-2 homolog, and Y1686, perhaps binding to SEM-5 (Fig. 2A) (Songyang et al., *Cell* 72: 767-778, 1993). While mutations in, for example, ras and MAP kinase have not been identified in screens for dauer constitutive or dauer defective mutations, these general signaling pathway proteins may couple to DAF-2 as they couple to insulin signaling in vertebrates (White and Kahn, *J. Biol. Chem.* 269: 1-4, 1994). The predicted phosphotyrosine residues in juxtamembrane

region and the kinase domain activation loop are circled. In the extended C-terminal region, predicted phosphotyrosine residues are also circled and SH2-binding sites are underlined (see below).

Amend the paragraph beginning at page 36, line 13, as follows.

Fig. 2B shows the cDNA encoding the *C. elegans* DAF-1 (SEQ ID NO:11).

Amend the paragraph beginning at page 36, line 14, as follows.

Fig. 2C shows the amino acid comparison of *C. elegans* DAF-2 (SEQ ID NO:12) to the human insulin receptor and human IGF-I receptor (shown in parenthesis) (SEQ ID NOS:103 and 104), and to the *Drosophila* insulin receptor homolog (SEQ ID NO:105), with *daf-2* and human insulin receptor mutations highlighted. Six *daf-2* mutations map in the ligand-binding domain: *sa187* (C347S, TGT to AGT), *e1368* (S451L, TCA to TTA), *e1365* (A458T, GCT to ACT), *sa229* (D526N, GAT to AAT), and two mutations in *mg43* (C279Y, TGT to TAT and P348L, CCC to CTC). Three *daf-2* mutations substitute conserved amino acid residues in the insulin receptor kinase domain: *sa219* (D1252N, GAT to AAT), *e1391* (P1312L, CCC to CTC), and *e1370* (P1343S, CCA to TCA). Darkened residues indicate amino acid identity. Hatched residues indicate amino acid similarity. The percentages under the domains represents the percentage of identity observed between DAF-2 and each receptor. The corresponding BLAST probabilities of

DAF-2 random match to each protein is: 6.4 x 10^{-157} (human insulin receptor), 2.7 x 10^{-156} (human IGF-I receptor), 2.1 x 10^{-153} (molluscan InR homolog), 8.3 x 10^{-153} (mosquito InR ~~homolgoue~~ homolog), 1.6 x 10^{-138} (human insulin receptor-related receptor), 1.7x 10^{-122} (*Drosophila* InR homolog), 2.0 x 10^{-108} (Hydra InR homolog). DAF-2 is more distant from the next most closely related kinase families: 8.9 x 10^{-58} (v-ros) and 3.0 x 10^{-51} (trkC neurotrophin receptor).

Amend the paragraph beginning at page 40, line 8, as follows.

Fig. 5C shows the protein sequence alignment of *C. elegans daf-3* (SEQ ID NOS:111 and 113) and the closest homolog found to date, human DPC4 (SEQ ID NOS:112 and 114), in the Smad conserved domains I and II. Dots indicate gaps introduced to maximize alignment. DAF-3 is 55% identical to DPC4 in domain I and 30% identical in domain II. *daf-3*(mg125) and *daf-3(mg132)* mutations are indicated by boldface and underline. The Smad mutational hotspot is underlined. In addition to *mg125* and *mg132*, seven other *daf-3* alleles were sequenced in the hotspot; none of them contains a mutation. Alleles sequenced were *mg91*, *mg93*, *mg105*, *mg121*, *mg126*, *mg133* (isolated by A. Koweek and G. Patterson, unpublished) and sa205.

Amend the paragraph beginning at page 43, line 10, as follows.

Fig. 21A ~~(SEQ ID NOS:211-215)~~ is an illustration showing that human FKHR

(SEQ ID NO:57), FKHRL1 (SEQ ID NO:330), and AFX (SEQ ID NO:331) are the closest relatives to DAF-16 (SEQ ID NO:45). Note that the differentially spliced DAF-16 forkhead domain (SEQ ID NO:329) is less homologous.

Amend the paragraph beginning at page 44, line 23, as follows.

Fig. 25 is an illustration showing the comparison of *C. elegans* AKT with mammalian AKT (SEQ ID NOS:87-102, 325, and 326).

Amend the paragraph beginning at page 44, line 5, as follows.

Fig. 28 is a graph illustrating the homology of *C. elegans* insulin-like molecules (SEQ ID NOs:117-124) with human insulin (SEQ ID NO:125) and a consensus motif (SEQ ID NO:324).

Amend the paragraph beginning at page 46, line 24, as follows.

Figs. 39A and 39B illustrate that *daf-18* encodes a homologue of PTEN (MMAC/TEP1). Fig. 39A shows the exon/intron structure of DAF-18 (~~SEQ ID NO:365-368~~ SEQ ID NOS:307, 327, and 328). The phosphatase domain is indicated in gray. The bottom of this figure indicates that *daf-18(e1375)* has a 30 base pair insertion in the fourth exon. 13 base pairs (shaded) are duplicated along with two smaller segments of the repeat (thick bars). This mutation introduces a premature stop codon (*). Fig. 39B shows an alignment of the phosphatase domains of DAF-18 and PTEN (GeneBank accession

U93051) (~~SEQ ID NO:369-378~~ SEQ ID NOS:308 and 309). Pileup (GCG) was used to align the entire coding sequence. The phosphatase domain is shown with identical amino acids shaded. The probable active site Cys-$(X)_5$-Arg sequence is indicated with a bar.

Amend the paragraph beginning at page 47, line 10, as follows.

Figs. 40A and 40B show the amino acid and nucleic acid sequences of the *C. elegans daf-18* gene (~~SEQ ID NO:379-380~~ SEQ ID NOS:310 and 311).

Amend the paragraph beginning at page 47, line 25, as follows.

Fig. 42 shows the *C. elegans cod-5* nucleic acid and amino acid sequences (~~SEQ ID NO:381-382~~ SEQ ID NOS: 312 and 313).

Amend the paragraph beginning at page 48, line 2, as follows.

~~**Figs. 43**~~ **Fig 43** shows the *C. elegans cod-5* knockout cDNA and amino acid sequences (~~SEQ ID NO:383-384~~ SEQ ID NOS:314 and 315).

Amend the paragraph beginning at page 50, line 11, as follows.

Figs. 47A and 47B show the nucleic acid and amino acid sequences of a human DAF-7 homologue (~~SEQ ID NO: 385-386~~ SEQ ID NOS: 316 and 317).

Amend the paragraph beginning at page 95, line 8, as follows.

The present model, based on genetic evidence that Akt/PKB couples insulin

receptor-like signaling to transcriptional output via the DAF-16 Fork head transcription factor in *C. elegans*, predicts that Akt/PKB will have transcriptional outputs in insulin-like signaling across phylogeny. It was previously suggested that the human homologs of the DAF-16 transcription factor (AFX, FKHR, FKHRL1 and AF6q21) may be the pertinent downsteam effectors of insulin signaling in humans (Ogg et al., *Nature* 389:994-999, 1997). Two of the consensus Akt/PKB sites conserved in DAF-16 and its human homologs are located outside of the Fork head DNA binding domain, and two sites are located in the highly basic W2 region of the Fork head domain that has been shown to mediate DNA phosphate backbone contacts (Clark et al. (1993) Nature 364:412-420). Insulin stimulated Akt/PKB phosphorylation of the W2 sites may affect DNA binding whereas the other conserved sites may affect transactivation. A recent report shows that Akt/PKB mediates insulin dependent repression of the insulin-like growth factor binding protein-1 (IGFBP-1) gene in HepG2 cells via a conserved insulin response sequence (CAAAAC/TAA) (SEQ ID NO:318) (Cichy et al., *J. Biol. Chem.* 273:6482-6487, 1998). Interestingly, we have determined that DAF-16 binds to this same insulin response sequence *in vitro*. We propose that Akt/PKB mediates its transcriptional effects on insulin responsive genes such as IGFBP-1 via the human homologs of DAF-16: AFX, FKHR, FKHRL1, or AF6q21.

Amend the paragraph beginning at page 96, line 15, as follows.

From the same genetic screen that generated the *akt-1(mg144gf)* allele, we identified another *age-1* suppressor, *mg142*. This mutation also bypasses the need for upstream *age-1* signaling and is genetically dominant. Genetic mapping placed the mutation in the region where a *C. elegans* homologue maps. The genomic sequence of *pdk-1*, starting 60 bp upstream of the start codon and ending 60 bp downstream of the stop codon is shown in Figure 35 (SEQ ID NO: 158). Figures 36 and 37 show the two *C. elegans pdk-1* spliced forms, *pdk-1a* (Figure 36; SEQ ID NO: 159) and *pdk-1b* (Figure 37; SEQ ID NO: 160). The *pdk-1(mg142)* gain of function mutation is Ala303Val (splice 1). This protein is 58% identical to mammalian PDK in the plecstrin homology domain and 39% identical in the kinase domain as shown below (~~SEQ ID NOS: 170-199~~ SEQ ID NOS:170-201).

Amend the paragraph beginning at page 120, line 15, as follows.

We have constructed a full length protein fusion of GFP to a highly expressed glucose transporter orthologue in the worm genome: H17B01. The H17B01.1 (GLUT) GFP fusion was amplified with primer CAW59 (ccactatggccgagatttcc) (SEQ ID NO: 319) and CAW60 (ccagtgaaaagttcttctcctttcttcctcttctcgaattcgga) (SEQ ID NO: 320). CAW 59 is the promoter primer and corresponds to nucleotides 31101-31120 in cosmid H17B01 and 39249-39268 in YAC Y51H7.contig253. Primer CAW60 is the GFP-fusion primer. The

first 23 nucleotides are GFP and the last 21 are GLUT bottom strand (i.e., cttcctcttctcgaattcggc) (SEQ ID NO:321) corresponding to 48128-48108 in Y51H7.contig253 and 5015-5035 in C13F7 (the cosmid that joins H17B01). The protein sequence is as follows (SEQ ID NO: 208):

Amend the paragraph beginning at page 177, line 22, as follows.

Shown below are conserved protein regions of *C. elegans* homologues of key metabolic enzymes ~~SEQ ID NOS: 211-363~~ SEQ ID NOS:211-303). GFP fusions may be constructed using the 5' promoter regions located between these conserved protein domains and the next gene located 5' to these regions, as described above for the glucose transporter GFP fusion gene.

Amend the paragraph beginning at page 212, line 7, as follows.

DAF-7 (9 amino acid motif) (~~SEQ ID NO:364~~ SEQ ID NO:304).

GWDXXIAPK

Amend the paragraph beginning at page 213, line 3, as follows.

To date, the closest homologues of *C. elegans* appear to be members of the vertebrate GDF-8 and GDF-11 gene family, with a representative homologue shown in Figures 47A and 47B. These human proteins, whose composition and function in muscle

size determination have been described (McPherron AC, Lee SJ, Proc Natl Acad Sci U.S.A.1997 Nov 11;94(23):12457-61), may also function in metabolic control in conjunction with insulin. Alterntaively, there may be more than one DAF-7 orthologue, or a closer relative to DAF-7 in mammalian databases that subserves the metabolic role, whereas GDF-8,11 serve related roles in muscle control. The DAF-7 gene does not appear in worm EST databases, most likely because it is expressed in a single neuron, a very low expression level. Even though the mammalian EST databases are about 10 fold larger than the *C. elegans* EST base, if human DAF-7 is expressed in a small set of neurons, it is not surprising that it has not yet been seen in the EST database. Nonetheless, human DAF-7 may be instantly recognized using the motif, GWDXXIAPK (SEQ ID NO:304) as a means to search updated sequence databases or by standard techniques as described herein.